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Efficacy evaluation of the herbal solution on atopic dermatitis in goats

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Abstract

The herbal solution containing onion (*Allium cepa*), neem (*Azadirachta indica*) Lemon (*Citrus limon*) and Aloe vera (*Aloe barbadensis miller*) was prepared. The effect of the said herbal solution on alleviating symptoms of atopic dermatitis in surti goats was studied. Among 12 atopic dermatitis affected goats; 6 were treated with the herbal solution daily for 30 days while 6 goats were kept as control whereby no treatment was given. The treatment with the herbal solution was found to have curative effects on atopic dermatitis in terms of reduced severity of clinical signs, reduced severity of histopathological lesions in the skin biopsies, reduced load of yeast in skin scraping examination and overall relief in the clinical signs.

Keywords: goats, atopic dermatitis, herbal, histopathology, clinical signs

Introduction

Atopic dermatitis has many aetiologies comprising of environmental, nutritional, genetic and parasitic entities. The causes and pathogenesis of atopic dermatitis are not well established but, it was believed to be due to too many reactive inflammatory cells in the skin and immunologic aberrations. Goats with atopic dermatitis are believed to be genetically predisposed to become sensitized to allergens in the environment [1]. Atopic dermatitis in goats can cause alopecia, scaling, crusting and red rashes on the body and such animals are prone to be further complicated by secondary bacterial infections [2, 3, 4]. Herbal therapy for skin and hair infections have an important role in the veterinary field. The combination of herbal agents like an onion (*Allium cepa*), neem (*Azadirachta indica*), Lemon (*Citrus limon*) and Aloe vera (*Aloe barbadensis miller*) may have an efficient therapeutic effect to counteract hair and skin damage. In the context of the same, we have formulated a solution having herbal and synthetic agents and evaluated its effect on hair follicles and skin epidermal infections in surti goats. The present study addresses the effect of the herbal solution on histopathological changes in the skin of atopic dermatitis affected goats of the surti breed. The study was carried out at the field unit of surti breed goats of Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, India.

Material and Method of formulation

The herbal solution was prepared by a combination of items/reagents as in Table 1.

Table 1: Ingredients for the herbal solution.

Step No.	Items/ chemicals
1.	Cocoamido propyl Betaine
2.	Aloevera pulp liquid (<i>Aloe barbadensis miller</i>)
3.	Neem (<i>Azadirachta indica</i>) leaves extract
4.	Sodium lauryl ether sulphate
5.	Vitamin E
6.	Jasmine flavour
7.	Onion Extract (<i>Allium cepa</i>)
8.	Lemon juice (<i>Citrus limon</i>)
9.	Demineralised Water
10.	Baking soda

Sodium Laureth Sulphate also known as Sodium Lauryl Ether Sulphate (SLES) is an anionic detergent and surfactant and is also an effective foaming agent. Being a surfactant SLES helps in lowering the surface tension between the ingredients of the product. Cocamidopropyl betaine (CAPB) is an amphoteric synthetic detergent. CAPB also serves as an antistatic agent in hair conditioners, which most often does not irritate skin or mucous membranes. Lemons (*Citrus limon*) are rich in citric acid along with other vitamins and minerals like calcium and potassium which are beneficial for skin and hair. Lemons were cut and pressed to squeeze the juice. The so made juice was sieved through cloth to remove debris and filtrate lemon juice was used. Neem (*Azadirachta indica*) leaf extract has antifungal properties that may help in the treatment of dandruff. The antimicrobial property of neem can relieve the itchiness, inflammation and irritation associated with dandruff. The regenerative properties of neem help in reducing hair fall. Vitamin E is an antioxidant and proven agent to help in normal epithelisation and protect the epithelial surface damage. Aloe vera (*Aloe barbadensis miller*) is a well-known herb having antidandruff activity. Onions (*Allium cepa*) are known to have the ability to promote collagen production, which leads to the production of healthier skin cells. The onion skin mixture was created by combining the water and the onion skin. The onion skin mixture was boiled and stirred for at least 6 minutes, then simmered on low to medium heat, and stirred about 6 times for approximately 15 minutes.

Experimental protocol

To achieve the goals of the experiment; 12 female surti breed goats (Does) with atopic dermatitis symptoms having alopecia, scaling, crusting and red rashes on the body and ear were identified. Among these goats; 6 goats were considered as the control group (T1). No treatment was given to group T1 goats. The other 6 goats were engaged as a treatment group. The control and treatment group goats were kept in separate enclosures under daily observation for 30 days. All goats had ad libitum access to food and water.

Each goat from the treatment group was washed with plain water. About 15 gm of above prepared herbal solution was taken in the hand and poured on all over the body coats drop

by drop. The solution was hand rubbed on the body coats of the goats for 10 minutes and left undisturbed for 20 minutes. Later on, the body coats were rinsed off using clean water and dried thoroughly with a towel. The above treatment was done on treatment group goats daily basis for 30 days.

Observations

1. Clinical signs

Daily observations were made on each goat of both the groups (i.e Control and treatment groups) mainly focusing on alopecia, scaling, crusting, red rashes and itchiness.

2. Skin scraping samples

Skin scraps were taken from all the animals of both groups on day 30. Gram stains were performed on smears prepared from skin scrapings.

3. Histopathology

The affected body parts from each goat were demarked and selected for taking skin biopsy on day 30 from both groups (i.e Control and treatment group). Skin biopsies were taken after shaving the selected area using a 10 mm diameter punch biopsy tool. The animals were kept under the effect of local anaesthesia for skin biopsy collection. The tissues were kept in 10% neutral buffered formalin till histopathology processing.

Skin biopsies were briefly washed with 10% phosphate-buffered saline (PBS) solution and serial 5 µm sections from all specimens were mounted on glass slides stained with haematoxylin and eosin (HE) and analyzed by light microscopy at 100x and 400 x. 3 sections of tissue were taken from each animal.

Skin sections were examined for cellular, structural and vascular changes. The final values were expressed as the qualitative intensity of the observations of the three sections. The qualitative intensity were marked as ++++ for severe lesions, +++ for moderate lesions, ++ for mild lesions and + for minimal lesions. The histopathological examination of dermal inflammatory cell infiltrations (neutrophils, mast cells, eosinophils and lymphocytes) were performed in five randomly selected fields at 400× magnification in one section taken from the skin.

Table 2: Skin scrapping and Histopathological changes on day 30.

Sr. No	Parameter	Group 1	Group 2
1.	Skin scrappings		
a.	No. of Malassezia	+++	+
2.	Epidermis		
a	Cellular layers from the basal lamina to the stratum granulosum	+++	++
b	Cells with hydropic degeneration	++++	++
c	Areas of transudation	++	+
d	Areas of eosinophilic infiltration associated with the presence of mites and/or burrows	++	+
e	Exocytosis focus of inflammatory cells associated with vasodilation and vascular neofornation	++	++
f	Cells with hyperchromatosis with karyorrhectic and pyknotic nuclei	++	+
g	Perivascular inflammation	++	+
2	Dermis		
a	Presence of eosinophils	++	+
b	Presence of lymphocytes	++	+
c	Presence of mast cells	+	+
d	Presence of neutrophils	+++	++

Results

1. Clinical sign

The goats of the control group showed pruritus throughout the disease and intermittently scratched themselves. In the most

severe clinical stages, infested goats showed scales, alopecia, crusts and mild inflammation of eyelids and greasy appearance of the ear (generalized signs). Patches or annular areas with redness of the skin of the ear were present. The

intensity of the above symptoms was reduced in the treatment group goats, whereby a greasy appearance on day 30 was absent.

2. Skin scraping

Clusters of poorly staining yeast-like structures with broad-based budding were observed. Yeasts were identified as *Malassezia* spp. based on their characteristic size, their shape (oval to footprint shape) and broad-based unipolar budding of daughter cells. The presence of yeasts were at more intense level in non treated (control group) goats as compared to treated group goats. The comparison is depicted in Table 2.

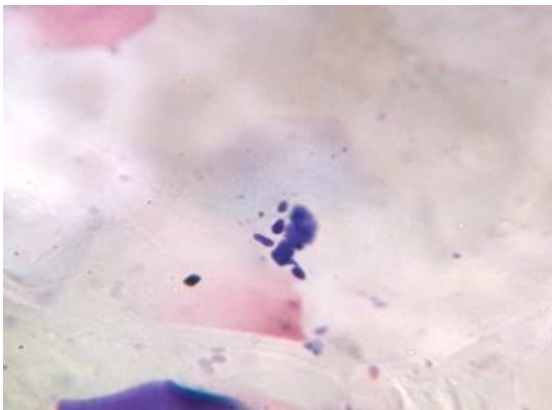


Fig 1: Malassezia in Skin scraping

3. Histopathological findings

Histopathological changes in atopic dermatitis affected skin between both groups were compared and shown in Table 2. Yeast was found within the stratum corneum of the epidermis. Malassezia infection could be due to overgrowth of normal flora resulting from disturbances of the normal physical, chemical or immunological mechanisms controlling the skin surface microenvironment and host defences [5, 6].

Hyperkeratosis was accompanied by epithelial hyperplasia and was characterized by thickening of the stratum corneum. Hyperkeratosis was mostly orthokeratotic (thickening of the cornified layer without retained nuclei and preserved keratinocyte maturation). Parakeratotic hyperkeratosis in the present study was characterized by the presence of nuclei in the cornified layer and concurrent with epithelial hyperplasia. Spongiosis was intercellular accumulation of fluid in the epidermis (Oedema). Acanthosis chiefly depicted the number of specialized squamous cells in the skin. Epithelial extensions projected into the underlying connective tissue in both skin and mucous membranes were also observed. Cellular injury lesions such as spongiotic oedema, apoptotic keratinocytes and exocytosis foci were qualitatively higher in control group goats as compared to treatment group goats.

Discussion

The macroscopic and histological skin lesions observed in atopic dermatitis goats closely matched the classical descriptions available in the existing literature. The cellular response was dominated largely by mononuclear cells, eosinophils and mast cells (intact and degranulated). The non treated affected goats showed signs that were resembling with a combined immediate and delayed type hypersensitivity reaction to infection [7]. In treated goats, these signs were of qualitatively lower intensity with cellular infiltrate which suggests cell-mediated immune reaction. The neutrophilic response could be both due to external agents such as yeasts

and bacterial infections. We can therefore say that the overexpression of eosinophils, mast cells and neutrophils were associated with a detrimental and non-protective response that was unable to control the infection. The presence of abundant serocellular crusts could be a protective mechanism against external parasitic agents [8].

Because of the opportunistic behaviour of *Malassezia* organisms, infection usually occurs when there are changes in the cutaneous environment or factors which alter the normal innate defence mechanisms [9]. The location of the study was at the hot and humid climate of South Gujarat (India) which is a predisposing factor for skin infections. Moreover, the present study was conducted in the rainy months of July, August and September. Looking at these facts, environmental changes such as humidity along with *Malassezia* could be the possible cause of atopic dermatitis in the present study. Predisposing factors may also include increased cutaneous relative humidity, changes in skin surface lipids, allergies, endocrinopathies, metabolic disorders, malnutrition and chronic glucocorticoid or other immunosuppressive therapy [5, 9, 10]. The reduced intensity of the clinical signs, skin lesions in histopathology and reduced presence of *Malassezia* yeast in the treated goats were indicative of potent healing/therapeutic effects of onion (*Allium cepa*), neem (*Azadirachta Indica*) and lemon (*Citrus limon*) with synthetic reagents.

Conclusion

Atopic dermatitis in the surti goats can be caused by many factors including environmental changes and yeasts as noticed in the present study. The combination of herbal agents like an onion (*Allium cepa*), neem (*Azadirachta Indica*) and lemon (*Citrus limon*) with synthetic reagents have a tremendous effect to counteract atopic dermatitis as evidenced by reduced severity of clinical signs, histopathological lesion of skin biopsies, reduced a load of yeast in skin scraping examinations and relief in terms of clinical signs.

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